

Pigment Analysis by HPLC at Horn Point Laboratory

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Pencil?! Who
uses pencil?

Is that 3625
or 3826??



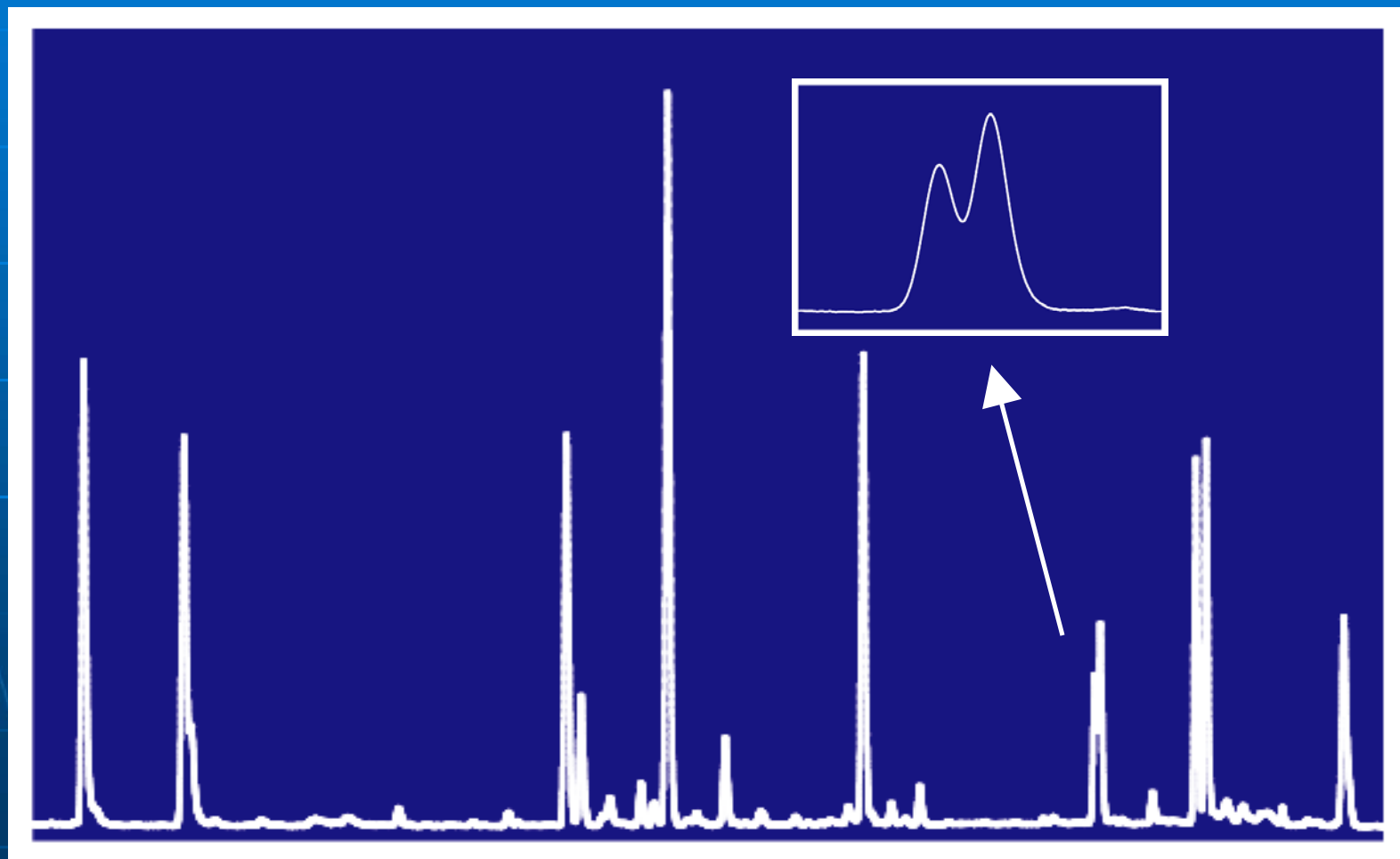
Emerging from the -25°C
freezer



Analysis procedures



Sample Chromatogram



Sample processing:

As of 10/30/06:

15% of samples analyzed

17% of time elapsed

Scheduling

- G. Fargion notifies PI for shipment date
- Samples to be completed by December:

	Received	HPLC analyzed	Data processed	Data reported
Chekalyuk	26	done	done	done
Mannino	356	done	done	done
Siegel	195	done	done	done
Mitchell	354	227	194	
Nelson	41			
Hill	296			
Subramaniam	300			
Total	1,568			

Topics of Discussion

- Quality Assurance at HPL
- Data reports from HPL

Quality Assurance at HPL

1. Method validation
2. Accuracy in Round-Robins
3. Continuous quality assessment

Method Validation

- Peak symmetry
- Resolution between peaks
- Quantitation in the presence of interferences
- Linear dynamic range
- LOD and LOQ
- Accuracy and precision
- Spiked recovery

Accuracy in Round-Robins

- SIMBIOS, SeaHARRE-1, -2, -3, and -4
- Reference values for natural samples are based on average consensus concentrations
- Accuracy is calculated as the % difference from the reference value
- Reference values should be determined from results of *quality-assured* laboratories

Calibration accuracy in Round-Robins

Average laboratory accuracy with standards

Round-Robin	HPL	Range (7 labs)	Overall average
SeaHARRE-2	1%	1 to 18%	7%
HPL-DHI (Spectrophotometric)	2%	--	--
HPL-DHI (HPLC)	2%	--	--

Continuous HPLC Quality Assessment at HPL

- Establish standardized procedures that limit uncertainties and blunders
- Perform Quality Control measurements during the analysis of samples
- Identify the range within which QC measurements should fall
- Take corrective action if QC measurement falls outside of expected ranges

The calculation equations

$$\tilde{C}_{P_i} = \hat{A}_{P_i} \times Rf$$

\tilde{C}_{P_i} = ng of pigment injected

\hat{A}_{P_i} = area of pigment

Rf = response factor

$$C_{P_i} = \frac{V_x}{V_f} \frac{\tilde{C}_{P_i}}{V_c}$$

C_{P_i} = $\mu\text{g/l}$ of pigment

V_x = extraction volume

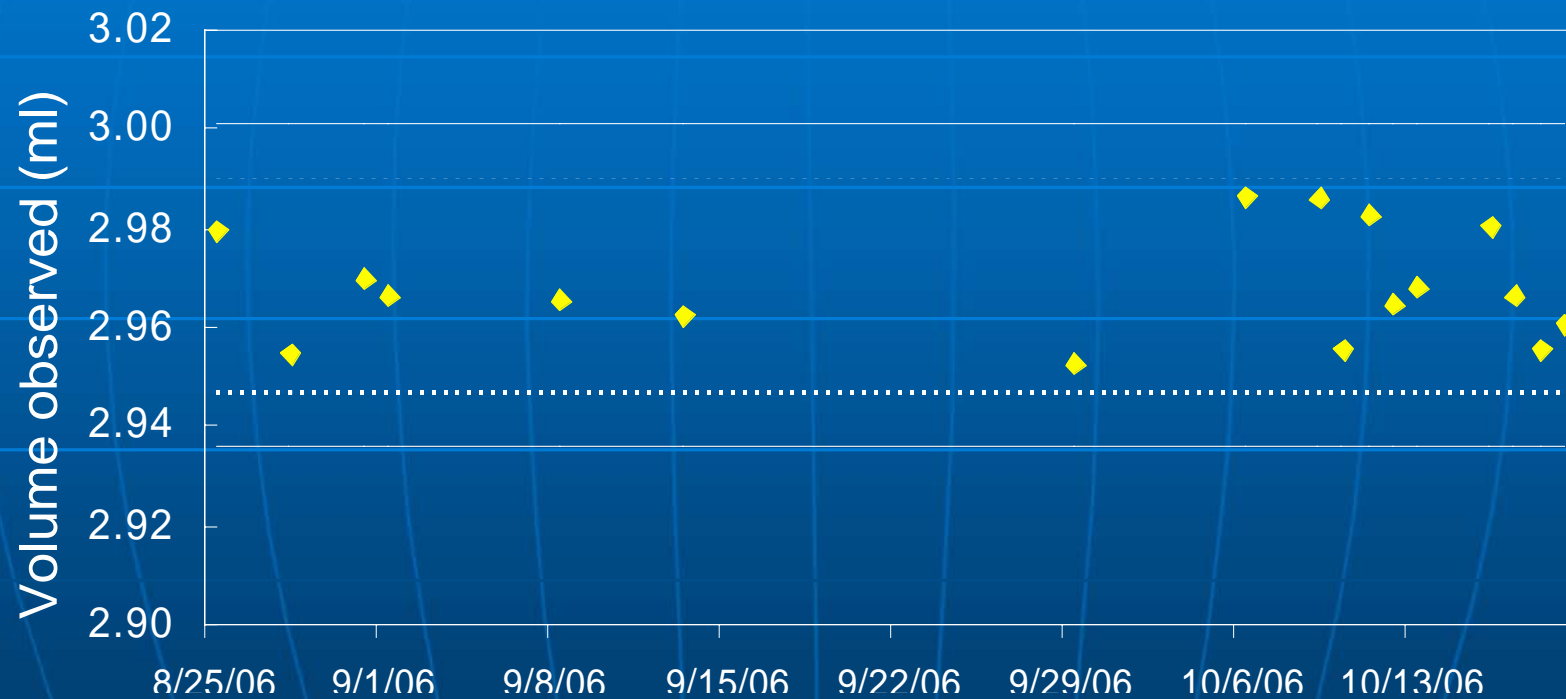
V_f = filtration volume

\tilde{C}_{P_i} = ng of pigment injected

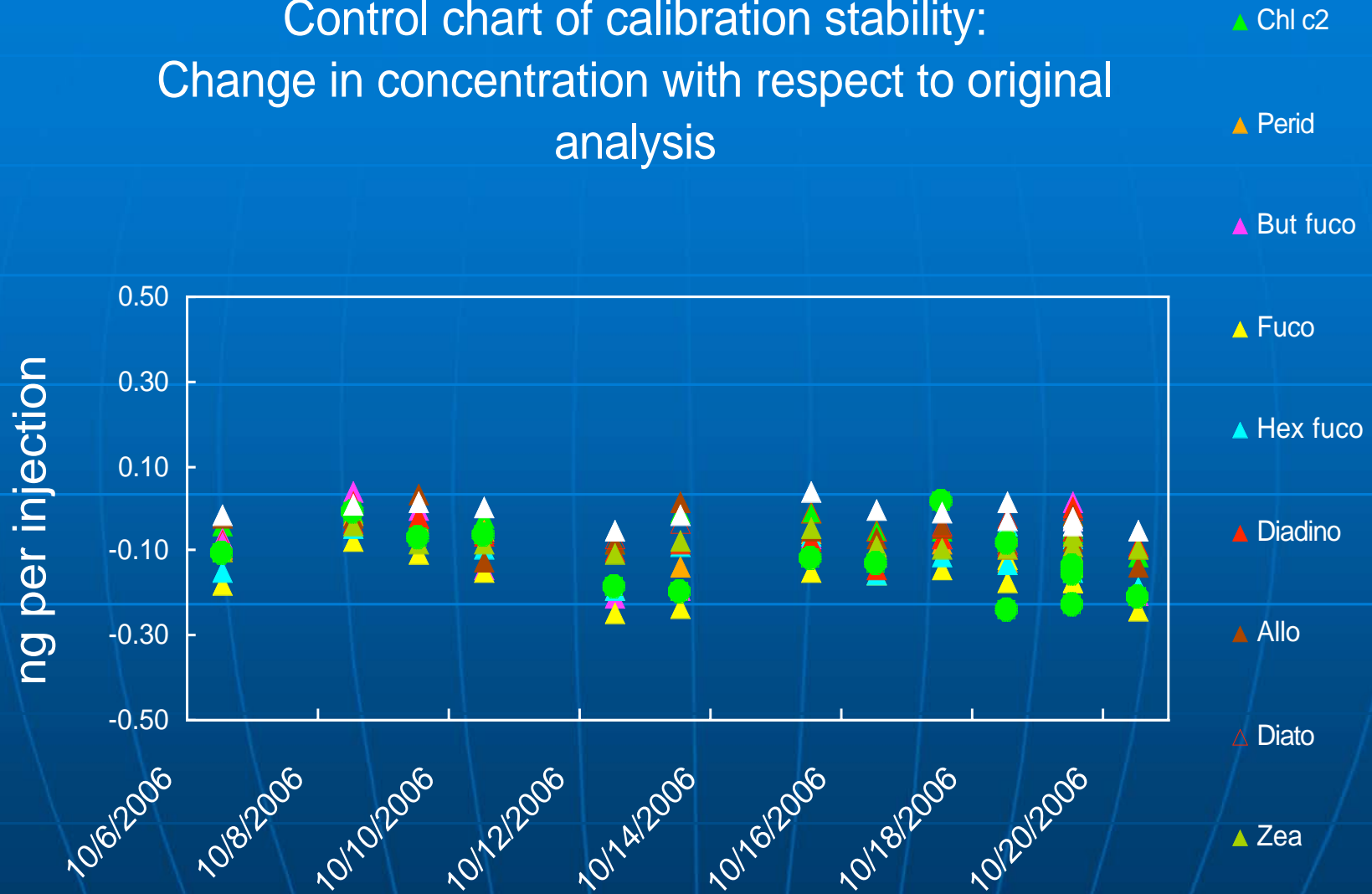
V_c = injection volume

Control chart

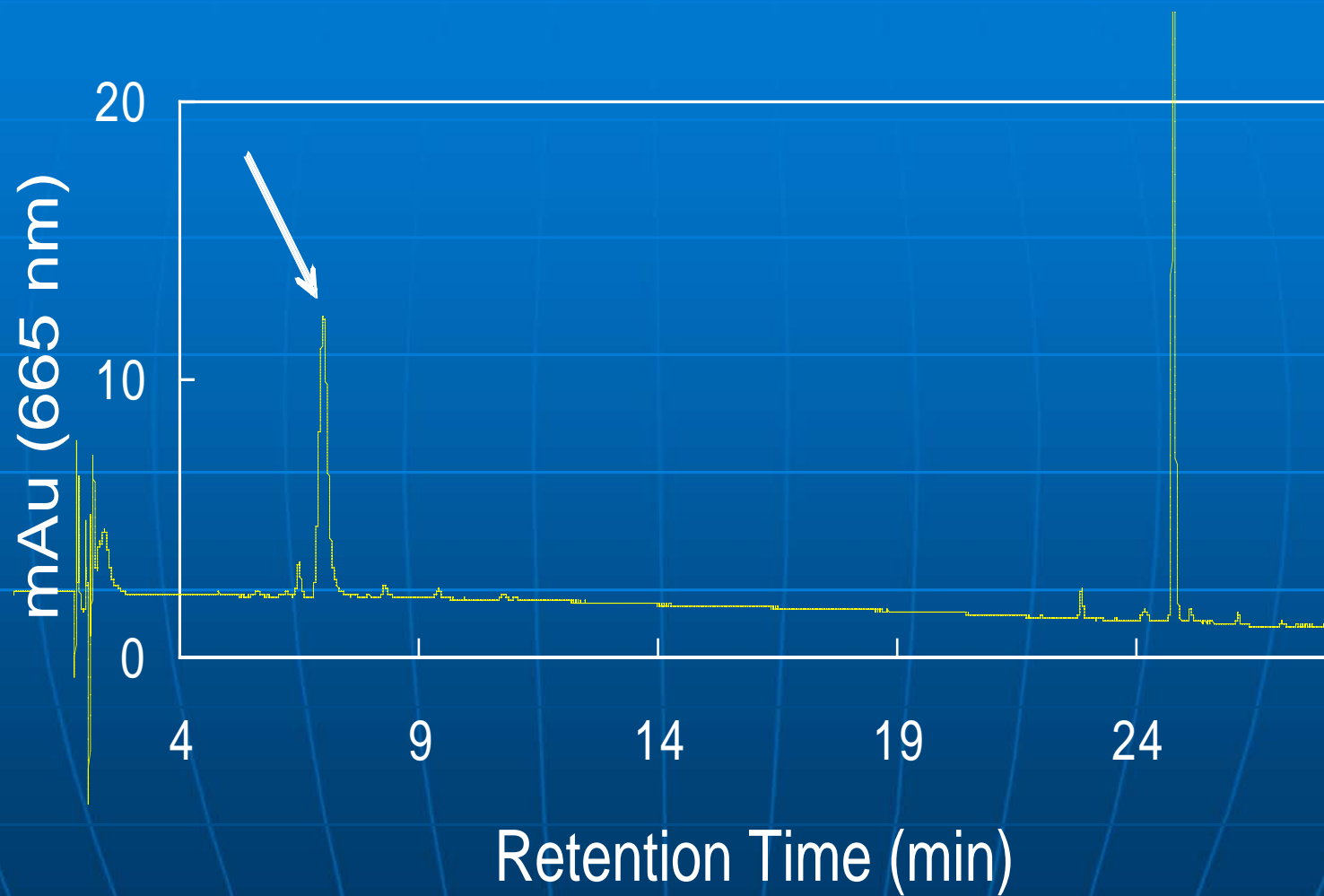
Repipette Calibration:
Setpoint volume = 3.00 ml



Control chart of calibration stability: Change in concentration with respect to original analysis



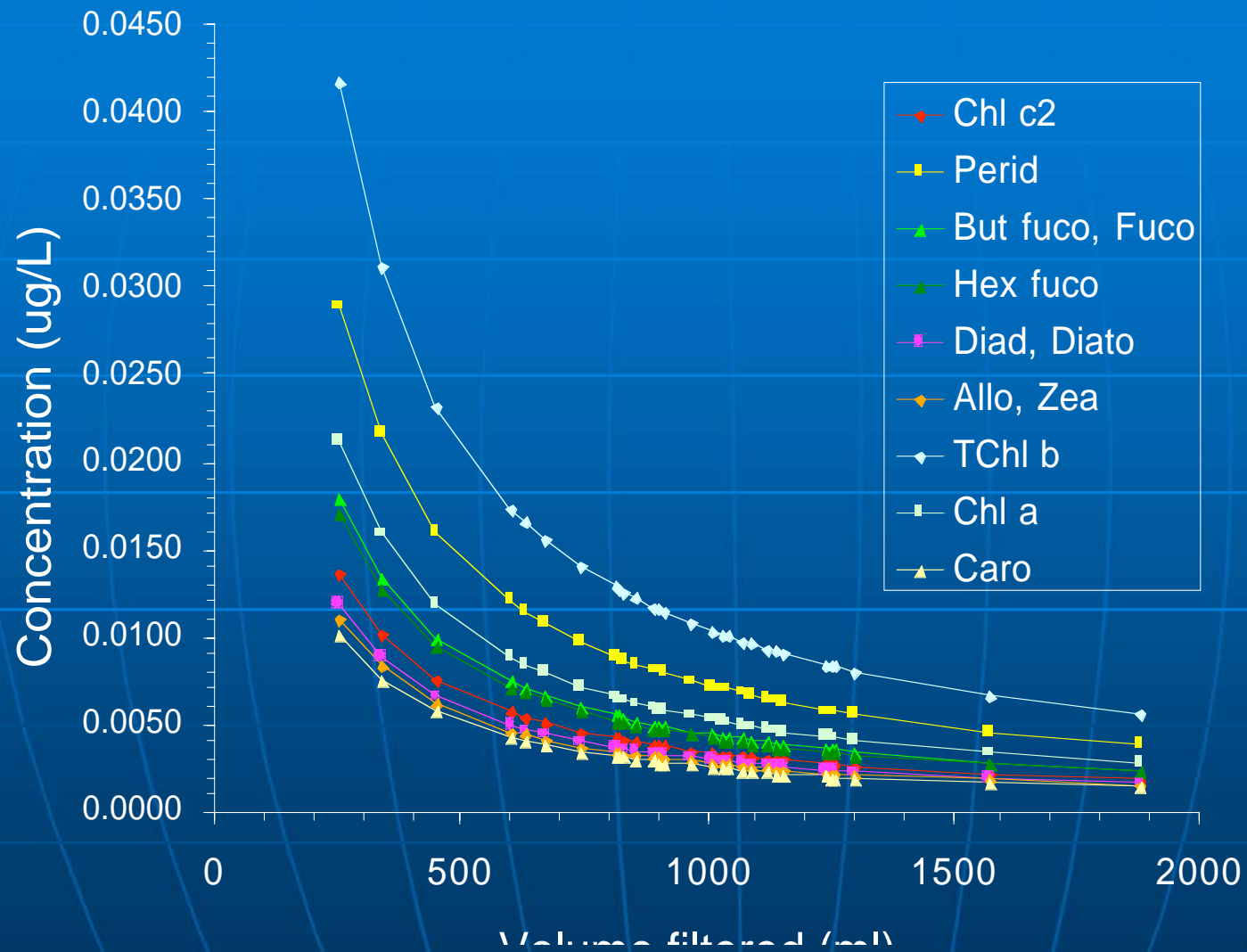
Blue felt pen



Data Reports from HPL

- Pigments not found and those with a SNR of 4 are given a concentration of 0.0001
- Graph of Effective LOQ (ug/L vs. filtration volume)
 - The ug/L of pigment in a sample for which the amount of pigment injected was at the instrument LOQ. Effective LOQ varies with changes in V_x and V_f .
 - LOQ = amount of pigment that results in a SNR of 10

Effective LOQ (extraction volume = 3.2 ml)



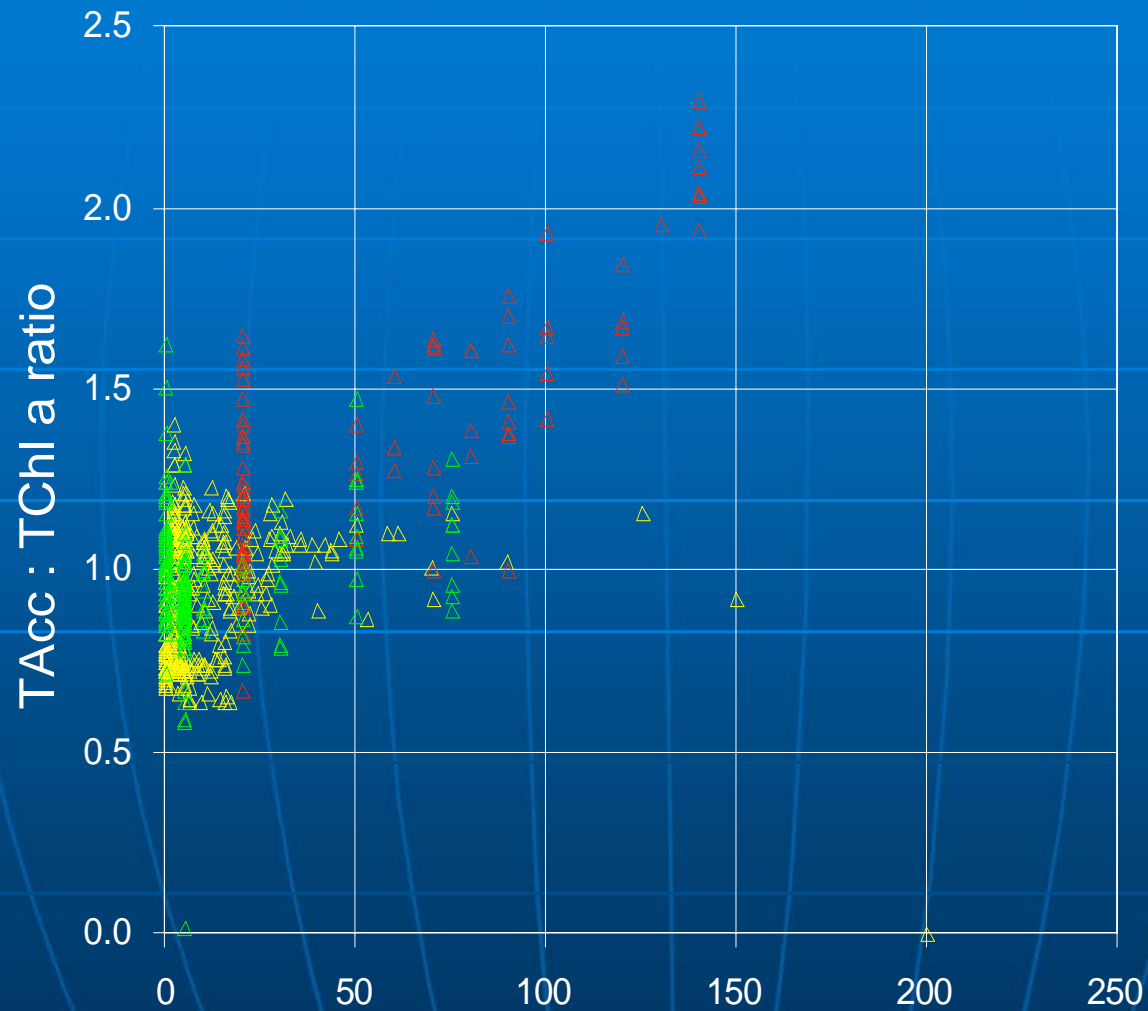
HPL sample codes

Horn Point Lab Sample Code	PI	Original PI Sample Code	Cruise Name	Sequential Sample Number
06-214	Siegel, David	DS	PB179	DS1948
06-214.5	Siegel, David	DS	PB179	DS1948
06-215	Siegel, David	DS	PB179	DS1949
06-216	Siegel, David	DS	PB179	DS1950
06-217	Siegel, David	DS	PB179	DS1951
06-218	Siegel, David	DS	PB179	DS1952

Duplicate injections of sample extracts



Pigment ratios (Trees et al, 2000)



Inter-method comparison at HPL

